

Description of a species of the genus *Paramesotriton* (Caudata, Salamandridae) from Guizhou, China, based on morphological and genomic evidence

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<https://zoobank.org/8D40ED2F-A2AB-463F-97D3-C97884C8BE1E>

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Academic editor: Umilaela Arifin ♦ Received 11 February 2025 ♦ Accepted 4 September 2025 ♦ Published 1 October 2025

Abstract

Species identification and delimitation are critical for biodiversity conservation. Recent mitochondrial-based phylogenetic studies of the genus *Paramesotriton*, a group of small salamanders inhabiting mountain streams in southern China and northern Vietnam, suggest that its diversity may be underestimated. In this study, we confirm and characterize a new species, *Paramesotriton wumengshanensis* sp. nov., using morphological, mitochondrial NADH dehydrogenase subunit 2 (ND2), mitogenome, and genomic evidence. This new species can be distinguished from other *Paramesotriton* species by characteristics such as rough and large body warts, large eyes, the absence of vestigial gills and gill filaments in adults, and distinctive vomerine teeth. Statistical analysis of the morphological data further showed that the new species is significantly different from its close relatives. Phylogenetic reconstruction based on concatenated and coalescent approaches, using both mitochondrial DNA and nuclear single nucleotide polymorphisms (SNPs) generated by restriction site-associated DNA sequencing, revealed that the new species forms a distinct lineage within the genus *Paramesotriton*, exhibiting a minimum genetic distance of 0.63% in mitochondrial ND2 compared to congeners. The observed cytonuclear discordance and minor mitochondrial differences among species may stem from historical gene flow and/or incomplete lineage sorting, and future studies using genomic evidence are needed to investigate their respective contributions. Based on this discovery, we recommend that independent evidence, especially genomic evidence, be integrated into the classification of closely related species to avoid falling into the trap of mitochondrial introgression.

Key Words

Guizhou, morphology, mitochondrial introgression, phylogenomics

Introduction

The Asian newts of the genus *Paramesotriton* Chang, 1935 (Caudata, Salamandridae) are among the most species-diverse caudate amphibians in China, second only to

the genus *Tylototriton* (AmphibiaChina 2025). Currently, 15 species have been recorded, which are divided into two species groups: the *P. caudopunctatus* group, which includes *P. caudopunctatus* (Liu & Hu, 1973), *P. longliensis* Li, Tian, Gu & Xiong, 2008, *P. malipoensis* Rao, Liu, Zhu

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& Ma, 2022, *P. maolanensis* Gu, Chen, Tian, Li & Ran, 2012, *P. wulingensis* Wang, Tian & Gu, 2013, and *P. zhijinensis* Li, Tian & Gu, 2008, and the *P. chinensis* group, which includes *P. aurantius* Yuan, Wu, Zhou & Che, 2016, *P. chinensis* (Gray, 1859), *P. deloustali* (Bourret, 1934), *P. fuzhongensis* Wen, 1989, *P. guangxiensis* (Huang, Tang & Tang, 1983), *P. hongkongensis* (Myers & Leviton, 1962), *P. labiatus* (Unterstein, 1930), *P. qixilingensis* Yan, Zhao, Jiang, Hou, He, Murphy & Che, 2014, and *P. yunwuensis* Wu, Jiang & Hanken, 2010 (Fei et al. 2006; Fei and Ye 2016; Rao 2022). These species are primarily distributed in southern China and northern Vietnam (Fei et al. 2006; Fei and Ye 2016; AmphibiaChina 2025).

The mitochondrial gene-based phylogeny identified two to three cryptic species within the *P. caudopunctatus* group (Luo et al. 2021; Luo et al. 2022). Luo et al. (2021) identified a *Paramesotriton* population in Dafang County, Guizhou Province, China, as a potential cryptic species, initially referred to as *Paramesotriton* sp2 (DF) and later as *P. longliensis* (DF) (Luo et al. 2022). However, this species was not formally described in either study. This cryptic species exhibits minimal mitochondrial variation (less than 2%) compared to currently recognized species, raising questions regarding its validity. This relatively small mitochondrial variation may be attributed to historical mitochondrial introgression. For instance, mitogenome introgression between *Odorrana grahami* and *O. junlianensis* in southern China led to minimal differences in mitochondrial cytochrome oxidase subunit I but significant nuclear gene divergence (Yuan et al. 2022). This highlights the importance of genotyping-by-sequencing to obtain thousands of nuclear single nucleotide polymorphisms (SNPs) for accurate species delimitation. Similarly, whole-genome resequencing has been employed to generate large SNP datasets, resolving the taxonomy of closely related species and identifying cryptic diversity (Hu et al. 2020; Yang et al. 2022; Gu et al. 2023). Thus, genome-level SNP data provided critical insights for resolving taxonomic uncertainties among closely related species, particularly those affected by mitochondrial introgression, and offered robust support for species delimitation.

Although our previous work using mitochondrial ND2 and combining phylogenetic and species delimitation methods identified the cryptic species *Paramesotriton* sp2 (DF) or *P. longliensis* (DF) (Luo et al. 2021; Luo et al. 2022), the smaller mitochondrial differences with closely related species make their validity not strongly supported. An additional problem is that while whole-genome resequencing can yield a large number of SNPs, the *P. caudopunctatus* group currently has no close reference genome that can be used. Therefore, we performed restriction site-associated DNA sequencing (RADseq), a reduced-representation genome sequencing technique (Miller et al. 2007; Andrews et al. 2016) that is versatile and does not require a reference genome, on 46 samples, thereby obtaining a large amount of SNP data. In this study, our aim is to confirm the validity of this cryptic species and formally describe it in combination with morphology.

Materials and methods

Sampling and morphological analyses

Among the 46 samples collected, nine were from *P. caudopunctatus*, 10 from *P. longliensis*, two from *P. maolanensis*, 10 from *P. zhijinensis*, eight from *Paramesotriton* sp2 (DF), and seven from the outgroup (Fig. 1, Suppl. material 1). Tissue samples and specimens of the remaining species were collected before 2018, except for *Paramesotriton* sp2 (DF), which was collected in July 2018. Muscle samples were collected from the tail tip and immediately preserved in 95% anhydrous ethanol, and specimens were fixed using 75% alcohol immersion and stored indoors in a refrigerator at -80°C . The field survey followed the rules of the Wildlife Protection Law of the People's Republic of China. All muscle samples and specimens were preserved in the Animal Ecology Laboratory, Guizhou Normal University, Guiyang, Guizhou Province, China. All animal experiments were approved by the Research Ethics Committee of Guizhou Normal University (approval number: 202504001).

In this study, we measured 15 morphological characters of 29 specimens using digital calipers accurate to 0.1 mm (Gu et al. 2012; Suppl. material 1), including new species (four males and three females), *P. longliensis* (eight males and nine females), and *P. zhijinensis* (five females). The measured characters were as follows: TOL = total length (from tip of snout to tip of tail); SVL = snout-vent length (from tip of snout to posterior edge of vent); HL = head length (from posterior edge of left parotoid to snout tip); HW = head width (maximum head width); SL = snout length (from tip of snout to the anterior corner of eye); TKL = trunk length (distance between axillae along right body side); IOD = interorbital distance (minimum distance between the eyes); ED = eye diameter (from the anterior corner to the posterior corner of the eye); TAL = tail length (from anterior tip of cloaca to tip of tail); TH = tail height (maximum tail height); TW = tail width (maximum tail width); FLL = forelimb length (from the base of the forelimb to the tip of the longest finger); HLL = hindlimb length (from the base of the hindlimb to the tip of the longest toe); DAG = distance between axilla and groin (from the posterior margin of the base of the forelimb to the anterior margin of the base of the hindlimb); and SW = snout width (maximum snout width). Principal component analysis (PCA) with eigenvalues greater than 1, the maximum variance method, and simple bivariate scatter plots were used to explore and characterize the morphometric differences between the new species and closely related species. A canonical discriminant analysis (CDA) was employed to distinguish between the new species and closely related species, as well as to predict group membership, following methods used in previous studies (Parsons and Jones 2000; Polaszek et al. 2004; Xiong et al. 2015; Shen et al. 2020). To reduce the effect of allometry, each morphometric measurement was corrected by dividing it by the TOL before conducting the

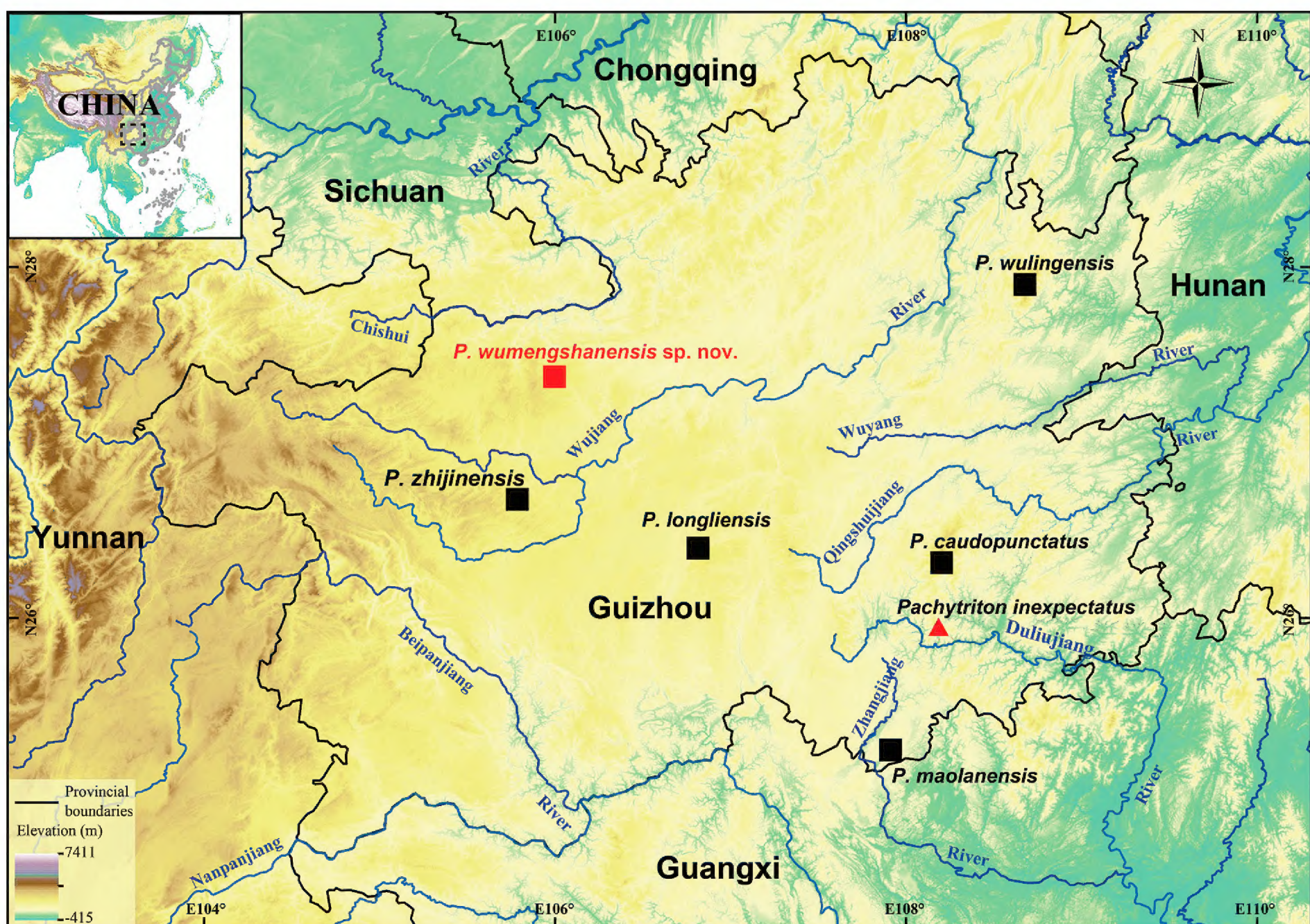


Figure 1. Distribution and sampling of the *P. caudopunctatus* group and outgroup *Pachytriton inexpectatus* in Guizhou, China.

PCA and CDA analyses. One-way analysis of variance (ANOVA) was conducted to determine the significance of differences in morphometric characters between the new species and the aforementioned closely related species. All statistical analyses were performed using SPSS v.21.0 (SPSS, Inc., Chicago, IL, USA), with differences considered statistically significant at $p < 0.05$. Morphological comparisons of the new species with congeners were based on specimen examination and literature.

Sex identification was performed based on Fei et al. (2006) and Fei and Ye (2016)—specifically, males possess a large and low cloaca, with a relatively long longitudinal anal fissure and finger-like papillae on the cloacal wall. In contrast, females have a small and high cloaca, a short oval-shaped anal fissure, and lack finger-like papillae on the cloacal wall.

Laboratory protocols, sequencing, and bioinformatic methods

The genomic DNA of 46 samples was extracted from 95% ethanol-preserved tissue using a standard CTAB protocol (Hanania et al. 2004). Genomic DNA from each sample was digested using the restriction enzyme EcoRI (GAATTC) following previously published procedures. DNA libraries for each sample were constructed according to the RAD protocol (Baird et al. 2008). PCR products

were purified to recover 350–550 bp DNA, and paired-end sequencing was conducted on the MGISEQ-2000. Raw reads were deposited in the NCBI Sequence Read Archive database (<https://www.ncbi.nlm.nih.gov/sra>) under the BioProject accession number PRJNA1016311.

Raw reads for samples were filtered using fastp v.0.23.4 (Chen 2023) with default parameters. Next, we performed further quality control on the cleaned reads using STACKS v.2.61 (Rochette et al. 2019). The process_radtags module was used to clean the tags by discarding low-quality reads, removing reads with uncalled bases, and rescuing barcodes and radtags. Additionally, we used the clone_filter module in STACKS to identify and remove PCR duplicates from the RADseq data. Single nucleotide polymorphism (SNP) calling for each sample was performed using the denovo_map.pl script in STACKS. We used the ustacks, cstacks, sstacks, tsv2bam, and populations modules from the STACKS pipeline to complete the de novo assembly of cleaned reads, generate consensus sequences, and perform SNP calling for each sample. To determine the optimal assembly parameters, we followed previous methods to evaluate two key parameters (Paris et al. 2017; Gundappa et al. 2022): (–M), the maximum number of nucleotide mismatches allowed between stacks, and (–m), the minimum depth of coverage required to create a stack. For our dataset, M ranged from 3 to 8 (in intervals of 1), while m ranged from 4 to 14 (values: 4, 6, 8, 9, 10, 12, 14). For the parameter n

(the number of mismatches allowed between sample loci when building the catalog) in the *cstacks* module, we followed the recommendation of Paris et al. (2017) and set $n = M$. Based on the r80 rule (Paris et al. 2017), the final optimal parameter combination was $M = 6$, $m = 10$, and $n = 6$. In total, the STACKS program identified 15,581,665 biallelic SNPs across 46 individuals.

In this study, we genotyped and filtered the samples to obtain reliable SNPs using the following process: (1) initial genotype filtering was performed based on genotype missing rate and minimum allele frequency using VCFtools v.0.1.16 (Danecek et al. 2011) with the following running parameters: `---maf 0.01 ---max-missing 0.5 ---min-alleles 2 --recode --recode-INFO-all --out`. (2) PLINK v.1.90 (Purcell et al. 2007) was then used to filter out samples with a missing genotype rate greater than 50%. (3) Missing genotypes were imputed using Beagle (Browning and Browning 2016). (4) Finally, the SNPs generated in the previous step were further filtered using the R package *bigsnpr* v.1.9.11 (Privé et al. 2018) based on linkage disequilibrium-based SNP clumping. In this study, only one SNP per locus was retained in the final SNP dataset.

Phylogenetic reconstruction and genetic differentiation based on SNP data

We used IQ-TREE v.1.6.12 (Nguyen et al. 2015) to reconstruct phylogenetic trees based on maximum likelihood for concatenated sequences from all loci to infer phylogenomic relationships. The analysis was performed with the following parameters: `-s --keep-ident --st DNA --nt --m GTR+ASC --bb 1000 --pre`. Node support values were assessed using the ultrafast bootstrap approximation (UF-Boot) method (Hoang et al. 2018). Species tree inference was performed using SVDquartets (Chifman and Kubatko 2014) implemented in PAUP* 4.0a (Wilgenbusch and Swofford 2003). SVDquartets estimates relationships between taxa under the coalescent model by inferring splits among quartets of randomly sampled taxa. All possible taxon quartets were evaluated, and node support was estimated using 100 standard bootstrap replicates. In cases where node support was low, the SVDquartets analysis helped identify potential incomplete lineage sorting or introgression among species. Both analyses used 1,475,246 unlinked SNPs. The fixation index (*F_{st}*) between the new species and its closely related species was assessed using VCFtools v.0.1.16 (Danecek et al. 2011) to quantify the degree of genetic variation among different species.

Phylogenetic reconstruction, genetic distance, and haplotype network based on mitochondrial sequences

The 48 mitochondrial ND2 sequences and 19 mitochondrial genomes used for phylogenetic reconstruction were primarily sourced from our previously published data

(Table 1) (Luo et al. 2021; Luo et al. 2022). Multiple sequence alignment was performed using MUSCLE (Edgar 2004) within MEGA v.7.0 (Kumar et al. 2016), with default settings. Based on the Bayesian information criterion, we evaluated the best-fit partitioning schemes and corresponding nucleotide substitution models for ND2 and mitochondrial genomes using PartitionFinder v.2.1.1 (Lanfear et al. 2017). Phylogenetic trees were constructed using both maximum likelihood (ML) and Bayesian inference (BI) methods. ML analysis was run in IQ-TREE v.1.6.1 (Nguyen et al. 2015) with the best model and 20,000 ultrafast bootstrap (UBP) replicates. The BI tree was reconstructed using MrBayes v.3.2.1 (Ronquist et al. 2012). Two independent runs were conducted in the BI analysis, each of which was performed for 5 million generations and sampled every 1000 generations. The first 25% of the samples were discarded as a burn-in. Nodes were considered well supported when the Bayesian posterior probability (BPP) was greater than 0.95 and the ML ultrafast bootstrap value (UBP) was greater than 95%. Uncorrected p-distances (1000 replicates) based on the ND2 gene were calculated using MEGA v.7.0 (Kumar et al. 2016). Additionally, we reconstructed the mitochondrial ND2 gene haplotype network using PopART v.1.7 (Leigh et al. 2015) based on the median-joining method (Bandelt et al. 1999).

Data availability statement

The datasets containing the accession numbers for the *P. caudopunctatus* group generated in this study are available as supplemental information. Original sequence reads of genome data are available at the National Center for Biotechnology Information (accession numbers SAMN37379588–SAMN37379633).

Results

Morphological differences based on measured data

We measured 15 morphological characters in 29 specimens (Suppl. material 1) and performed PCA, CDA, and ANOVA analyses to assess differences between *P. wumengshanensis* sp. nov. and closely related species. For males and females, three and three principal components were extracted, respectively. The first two components accounted for 72.87% and 73.84% of the total variation (Suppl. material 2). In the PC1 versus PC2 scatterplot, *P. wumengshanensis* sp. nov., *P. longliensis*, and *P. zhijinensis* formed nearly distinct clusters, with slight separation along the PC1 axis (Fig. 2A, B). For males, traits that loaded on the PC1 axis included total length, snout–vent length, head length, head width, eye diameter, tail width, forelimb length, and distance between axilla and groin (Suppl. material 2). For females, traits

Table 1. Localities, voucher information, and GenBank numbers for all samples used. For the voucher number and locality of the mitogenome, see Luo et al. (2022b).

ID	Species	Voucher number	Locality (* type localities)	ND2	Mitogenome
1	<i>P. longliensis</i>	GZNU 20070421001	Longli, Guizhou, China*	FJ169608	ON357921
2	<i>P. longliensis</i>	KIZ-GZH 081025	Longli, Guizhou, China*	GU980576	
3	<i>P. longliensis</i>	GZNU 20070421004	Longli, Guizhou, China*	JF438975	
4	<i>P. longliensis</i>	GZNU 20070421003	Longli, Guizhou, China*	JF438974	
5	<i>P. longliensis</i>	GZNU 20070421002	Longli, Guizhou, China*	JF438973	
6	<i>P. longliensis</i>	GZNU 2018061813	Huishui, Guizhou, China	MW147285	ON357922
7	<i>P. longliensis</i>	GZNU 2018061814	Huishui, Guizhou, China	MW147283	MW524141
8	<i>P. longliensis</i>	GZNU 20180618016	Huishui, Guizhou, China	MW147286	
9	<i>P. longliensis</i>	GZNU 20180618017	Huishui, Guizhou, China	MW147284	
10	<i>P. maolanensis</i>	GZNU 2006030003	Libo, Guizhou, China*	FJ169607	ON357924
11	<i>P. maolanensis</i>	GZNU 2006030006	Libo, Guizhou, China*	JF438972	
12	<i>P. maolanensis</i>	GZNU 2006030004	Libo, Guizhou, China*	JF438993	
13	<i>P. maolanensis</i>	GZNU 2006030005	Libo, Guizhou, China*	JF438994	
14	<i>P. wumengshanensis</i> sp. nov.	GZNU 20180709024	Dafang, Guizhou, China*	MT811029	MW524142
15	<i>P. wumengshanensis</i> sp. nov.	GZNU 2018070904	Dafang, Guizhou, China*	MT811030	ON357926
16	<i>P. wumengshanensis</i> sp. nov.	GZNU 2018070905	Dafang, Guizhou, China*	MT811031	
17	<i>P. wumengshanensis</i> sp. nov.	GZNU 2018070903	Dafang, Guizhou, China*	MT811028	
18	<i>P. zhijinensis</i>	GZNU 20070415002	Zhijin, Guizhou, China*	JF438976	ON357925
19	<i>P. zhijinensis</i>	GZNU 20070415003	Zhijin, Guizhou, China*	JF438977	
20	<i>P. zhijinensis</i>	GZNU 20070415004	Zhijin, Guizhou, China*	JF438978	
21	<i>P. zhijinensis</i>	GZNU 20070415001	Zhijin, Guizhou, China*	FJ169609	
22	<i>P. zhijinensis</i>	KIZ-GZH 081026	Zhijin, Guizhou, China*	GU980575	
23	<i>P. wulingensis</i>	GZNU 07072001	Jiangkou, Guizhou, China	FJ938040	ON357928
24	<i>P. wulingensis</i>	GZNU 2007071002	Jiangkou, Guizhou, China	JF438987	
25	<i>P. wulingensis</i>	GZNU 2007071004	Jiangkou, Guizhou, China	JF438989	
26	<i>P. wulingensis</i>	GZNU 08072603	Youyang, Chongqing, China*	JF438991	
27	<i>P. wulingensis</i>	GZNU 08072602	Youyang, Chongqing, China*	JF438990	
28	<i>P. wulingensis</i>	GZNU 2007071003	Youyang, Chongqing, China*	JF438988	
29	<i>P. wulingensis</i>	GZNU 08072604	Youyang, Chongqing, China*	JF438992	
30	<i>P. wulingensis</i>	KIZ 21898	Fanjingshan, Guizhou, China	KJ650055	
31	<i>P. wulingensis</i>	KIZ 21899	Fanjingshan, Guizhou, China	KJ650056	
32	<i>P. caudopunctatus</i>	GZNU 2007071001	Leishan, Guizhou, China*	FJ169606	ON357930
33	<i>P. caudopunctatus</i>	GZNU 200904251	Leishan, Guizhou, China*	MW147288	
34	<i>P. caudopunctatus</i>	GZNU 200904252	Leishan, Guizhou, China*	MW147287	
35	<i>P. caudopunctatus</i>	GZNU 2007072005	Leishan, Guizhou, China*	JF438986	
36	<i>P. caudopunctatus</i>	GZNU 2009042501	Leishan, Guizhou, China*	JF438983	
37	<i>P. caudopunctatus</i>	GZNU 20050727001	Leishan, Guizhou, China*	JF438985	
38	<i>P. caudopunctatus</i>	GZNU 20050727002	Leishan, Guizhou, China*	JF438984	
39	<i>P. deloustali</i>	Tissue ID: GZNU35	Hekou County, Yunnan, China	ON357931	ON357931
40	<i>P. guangxiensis</i>	Tissue ID: GZNU36	Shiwandashan, Guangxi, China*	ON357932	ON357932
41	<i>P. yunwuensis</i>	GZNU20201018003	Xinyi City, Guangdong, China*	ON357933	ON357933
42	<i>P. labiatus</i>	GZNU20210531001	Jinxiu County, Guangxi, China*	ON357934	ON357934
43	<i>P. fuzhongensis</i>	GZNU20070520002	Zhongshan, Guangxi, China*	ON357935	ON357935
44	<i>P. hongkongensis</i>	Tissue ID: GZNU37	Hong Kong, China*	ON357937	ON357937
45	<i>P. qixilingensis</i>	Tissue ID: GZNU38	Yongxin County, Jiangxi, China*	ON357938	ON357938
46	<i>P. aurantius</i>	GZNU20201018001	Jingning County, Zhejiang, China*	ON357939	ON357939
47	<i>P. chinensis</i>	Tissue ID: GZNU39	Ningbo City, Zhejiang, China*	ON357940	ON357940
48	<i>Pachytriton inexpectatus</i>	GZNU20180706001	Danzhai County, Guizhou, China	ON422325	ON422325

that loaded on the PC1 axis included total length, snout–vent length, snout length, trunk length, interorbital distance, tail length, forelimb length, hindlimb length, and distance between axilla and groin (Suppl. material 2). The CDA based on the 15 morphological traits clearly classified all individuals into specific taxa, with CAN1 and CAN2 explaining 87.2% and 12.8% of the total variance, respectively. Distinct clusters were formed along the CAN1 axis (Fig. 2C; Suppl. material 2).

Univariate ANOVA revealed significant morphological differentiation between the new species and its congeners

(Table 2). Among males, *P. wumengshanensis* sp. nov. and *P. longliensis* showed statistically significant differences ($p < 0.05$) in snout length, tail width, and snout width (Table 2). In contrast, *P. wumengshanensis* sp. nov. and *P. zhijinensis* exhibited significant morphological divergence across nine measured traits: total length, trunk length, interorbital distance, tail length, tail height, tail width, forelimb length, hindlimb length, and snout width ($p < 0.05$). Among females, *P. wumengshanensis* sp. nov. and *P. longliensis* showed significant differentiation in eight morphological characters (Table 2).

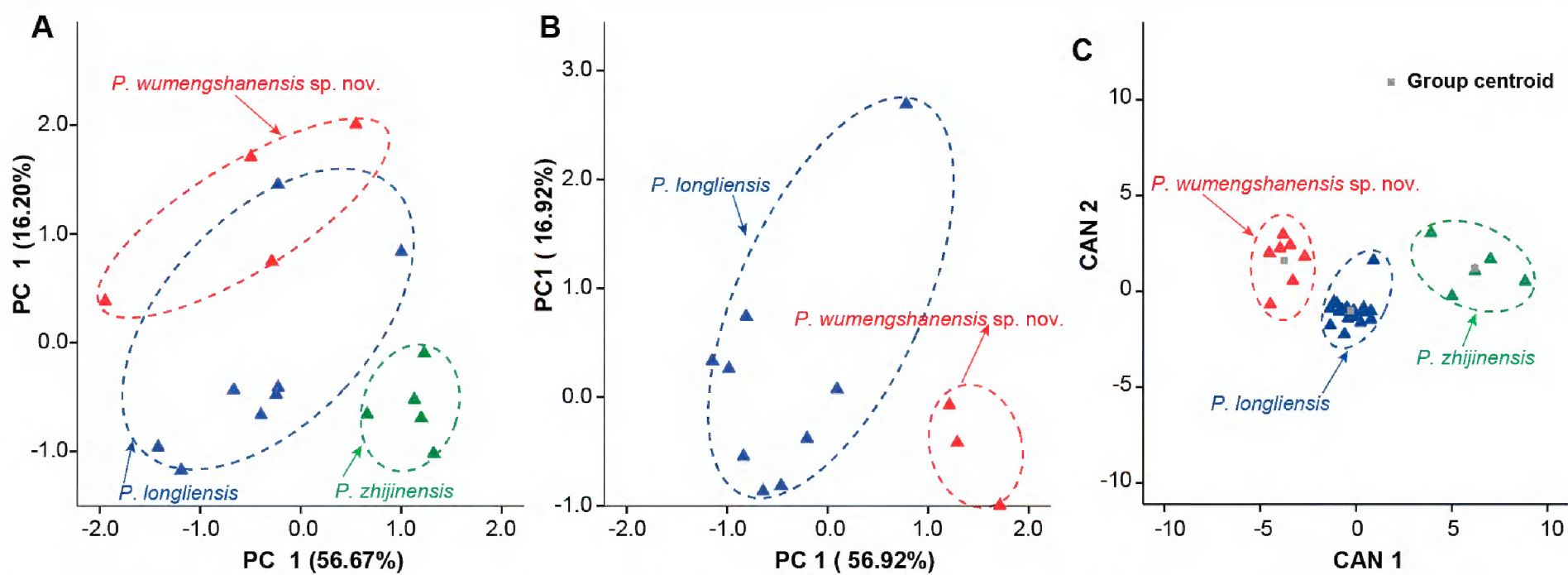


Figure 2. Plots of principal component analysis (A, B) and canonical discriminant analysis (C) scores of *P. wumengshanensis* sp. nov., *P. longliensis*, and *P. zhijinensis* based on morphological characters. A. Male; B. Female; C. Male and female.

Table 2. Morphometric statistics and results of analysis of variance from *P. wumengshanensis* sp. nov. (PW), *P. longliensis* (PL), and *P. zhijinensis* (PZ). All units are in mm. *P*-values are at 95% significance.

Measurements	<i>P. wumengshanensis</i> sp. nov.		<i>P. longliensis</i>		<i>P. zhijinensis</i>	P-value from ANOVA		
	Male (n = 4)	Female (n = 3)	Male (n = 8)	Female (n = 9)	Male (n = 5)	Male		Female
	Range (Mean ± SD)	Range (Mean ± SD)	Range (Mean ± SD)	Range (Mean ± SD)	Range (Mean ± SD)	PW vs. PL	PW vs. PZ	PW vs. PL
TOL	95.5–123.6 (110.2 ± 13.6)	150.1–160.0 (155.8 ± 5.1)	97.2–143.6 (117.0 ± 15.5)	114.6–136.3 (126.2 ± 8.4)	128.1–142.4 (136.7 ± 5.4)	0.610	0.014	0.013
SVL	53.0–72.6 (63.4 ± 8.9)	82.3–87.8 (85.6 ± 2.9)	47.7–78.3 (60.7 ± 9.9)	65.4–91.8 (71.5 ± 8.3)	63.7–68.3 (66.3 ± 1.8)	0.610	1.000	0.052
HL	16.5–23.9 (20.8 ± 3.3)	23.5–25.2 (24.6 ± 0.9)	16.3–22.1 (19.4 ± 1.8)	18.5–43.1 (22.7 ± 7.7)	20.9–23.9 (22.3 ± 1.1)	0.308	0.806	0.052
HW	11.8–18.9 (16.0 ± 3.3)	18.3–19.2 (18.7 ± 0.5)	12.5–16.6 (13.9 ± 1.5)	13.2–37.5 (17.8 ± 7.6)	15.1–17.9 (17.1 ± 1.1)	0.308	0.902	0.052
SL	6.3–8.6 (7.1 ± 1.1)	7.1–8.3 (7.9 ± 0.7)	5.7–7.6 (6.2 ± 0.6)	5.8–7.3 (6.5 ± 0.5)	7.0–8.5 (7.6 ± 0.6)	0.042	0.327	0.033
TKL	37.5–52.5 (45.6 ± 6.5)	61.0–66.5 (63.6 ± 2.7)	36.2–57.9 (46.7 ± 6.7)	46.9–59.8 (51.6 ± 4.3)	52.2–56.8 (54.6 ± 1.7)	0.865	0.027	0.013
IOD	4.5–6.8 (5.8 ± 1.1)	6.6–7.7 (7.3 ± 0.6)	4.5–7.7 (6.5 ± 0.9)	3.6–5.3 (4.4 ± 0.6)	8.3–9.5 (8.7 ± 0.6)	0.308	0.014	0.013
ED	2.9–3.9 (3.4 ± 0.5)	3.6–4.8 (4.1 ± 0.6)	2.3–3.8 (2.9 ± 0.5)	2.9–4.5 (3.6 ± 0.5)	2.7–3.6 (2.9 ± 0.4)	0.126	0.050	0.116
TL	40.8–55.1 (48.6 ± 6.1)	66.6–74.4 (70.0 ± 4.0)	45.8–63.8 (55.8 ± 6.1)	48.3–60.2 (54.7 ± 4.4)	63.8–71.9 (69.0 ± 3.5)	0.089	0.014	0.012
TH	6.3–9.9 (7.6 ± 1.6)	6.6–11.2 (9.1 ± 2.3)	6.7–10.0 (8.1 ± 1.0)	7.6–11.2 (9.0 ± 1.4)	8.4–20.0 (12.5 ± 4.5)	0.234	0.027	0.926
TW	2.4–3.3 (2.9 ± 0.4)	3.5–5.8 (4.7 ± 1.1)	3.4–5.1 (4.3 ± 0.5)	3.5–4.4 (3.9 ± 0.3)	4.5–7.3 (5.8 ± 1.1)	0.007	0.014	0.309
FLL	13.9–24.5 (18.4 ± 5.1)	26.0–28.2 (27.2 ± 1.1)	17.5–23.0 (20.4 ± 1.8)	19.4–23.6 (20.8 ± 1.3)	22.7–31.5 (27.5 ± 3.2)	0.497	0.027	0.013
HLL	14.8–26.2 (21.1 ± 4.8)	26.2–31.0 (28.0 ± 2.6)	17.5–27.1 (21.1 ± 2.9)	21.5–24.9 (23.2 ± 1.3)	24.2–30.9 (27.4 ± 2.4)	0.734	0.027	0.013
DAG	23.1–35.4 (29.9 ± 5.2)	41.3–41.9 (41.6 ± 0.3)	23.2–38.2 (28.7 ± 4.9)	23.0–40.5 (31.6 ± 5.7)	30.1–35.2 (32.2 ± 2.1)	0.865	0.462	0.013
SW	4.4–4.7 (4.6 ± 0.2)	4.1–5.8 (4.9 ± 0.9)	4.4–7.3 (5.5 ± 0.8)	4.1–5.8 (4.8 ± 0.7)	5.5–6.1 (5.8 ± 0.2)	0.027	0.014	0.926

Genetic data information

In this study, the full length of the mitochondrial ND2 used for phylogenetic reconstruction was 1407 base pairs (bp), of which 409 were variable and 277 were parsimony-informative. The model selection analysis suggested a division into three partitions, i.e., the first codon, the second codon, and the third codon of ND2, corresponding to the models HKY+G, HKY+I, and HKY+I. For the mitochondrial genome of 15,467 bp, the following evolutionary models were identified as optimal for the corresponding gene

segments: TVM+I+G for 12S rRNA and 16S rRNA, HKY+I+G for tRNAs, GTR+G for protein-coding genes (COX2, COX1, COX3, Cyt b, ATP8, ND2, ND3, ND4, ATP6, ND1, ND5, and ND4L), and HKY+G for ND6. A total of 46 samples generated approximately 2948.5 Gb of raw data and 1004 million paired-end reads, with an average of 64.09 Gb of data and 21.8 million paired-end reads per sample. A total of 15,581,665 biallelic SNPs were generated from the STACKS-cleaned reads. After filtering for low-quality individuals using PLINK, 1,475,250 SNPs remained.

Phylogenetic reconstruction, genetic distance, and haplotype

Phylogenetic reconstruction based on the mitochondrial ND2 gene and mitogenome revealed that *Paramesotriton* can be divided into Clade I and Clade II (Fig. 3A, B). Clade I corresponds to the *P. chinensis* group, with a large genetic divergence between the species, and the interspecific relationships are initially resolved. Clade II corresponds to the *P. caudopunctatus* group, which exhibits relatively low mitochondrial divergence yet encompasses six distinct evolutionary lineages (Fig. 3A). The four samples from Dafang, Guizhou, form a distinct evolutionary lineage within Clade II, which is close to *P. longliensis* and *P. maolanensis* (BPP/UFB = 0.84/72; Fig. 3A). However, the phylogenetic tree reconstructed from the mitogenome showed that the two Dafang samples from Guizhou clustered close to *P. longliensis* with low support (BPP/UFB = 0.47/42; Fig. 3B). The new species had small mitochondrial genetic distances from relatives, ranging from 0.63 to 9.39% (Table 3). The haplotype network based on ND2 showed no shared haplotypes within the *P. caudopunctatus* group, with the new species forming a unique haplotype (Fig. 3C).

Table 3. Uncorrected *p*-distance (%) between six species of the *P. caudopunctatus* group based on mitochondrial ND2.

ID	Species	1	2	3	4	5
1	<i>P. wumengshanensis</i> sp. nov.					
2	<i>P. caudopunctatus</i>	9.39				
3	<i>P. longliensis</i>	1.03	9.68			
4	<i>P. maolanensis</i>	0.63	9.49	0.61		
5	<i>P. wulingensis</i>	9.39	2.70	9.69	9.50	
6	<i>P. zhijinensis</i>	1.84	9.64	1.82	1.42	9.65

Using both concatenation and coalescent-based approaches, we reconstructed maximum-likelihood (ML) (Fig. 4A) and species trees (Fig. 4B) based on SNP data through IQ-TREE and SVDquartets analyses, respectively. Both analyses strongly supported the samples from Dafang, Guizhou, as a distinct evolutionary lineage. However, we observed phylogenetic discordance between the ML and species tree topologies regarding the placement of the new species and *P. zhijinensis*, suggesting potential influences of incomplete lineage sorting and gene flow events on phylogenetic reconstruction. The new species exhibits moderate genetic differentiation

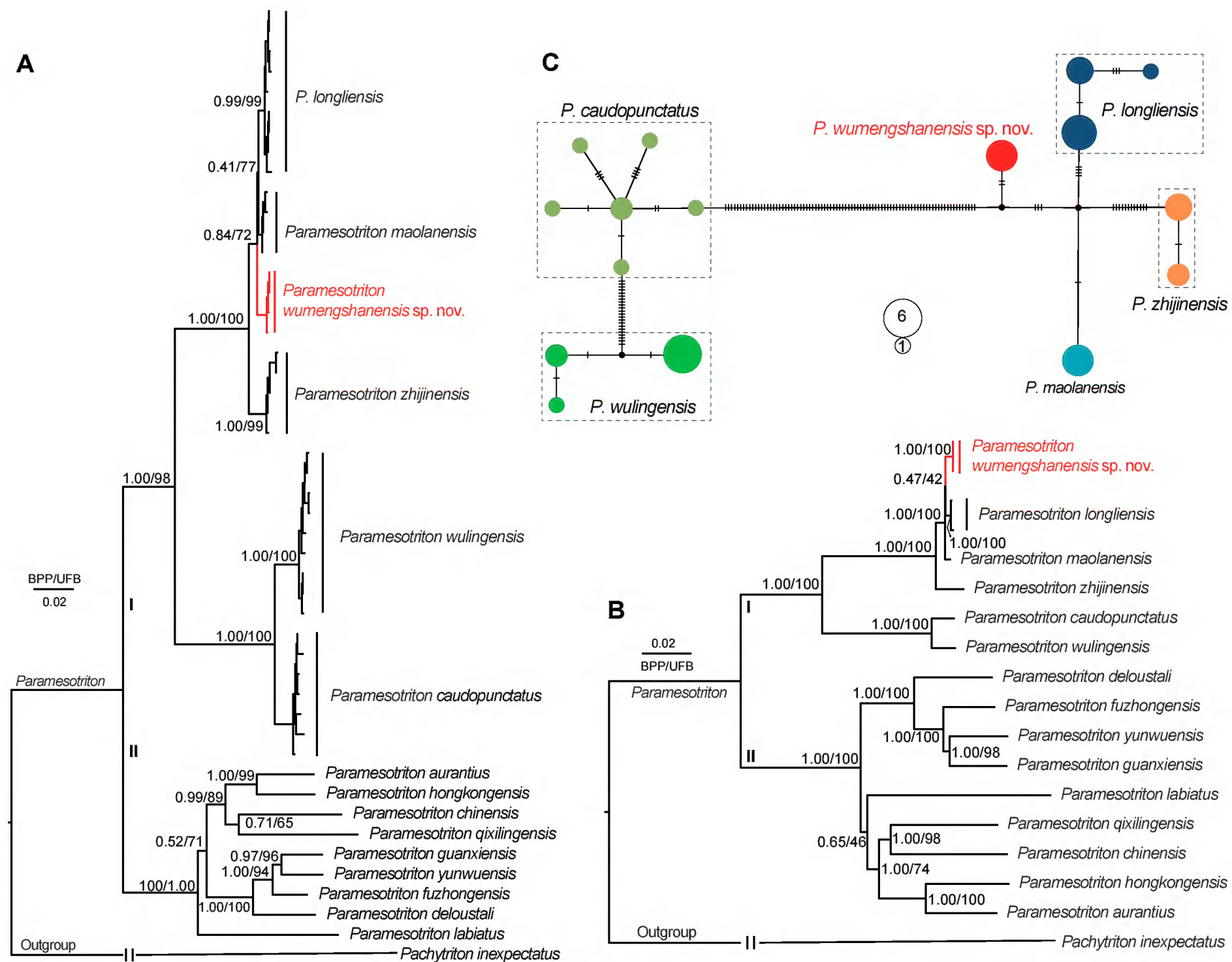


Figure 3. Reconstructed phylogeny and haplotype network. **A.** Phylogenetic tree reconstructed based on the mitochondrial ND2 gene; **B.** Phylogenetic tree reconstructed based on the mitochondrial genome. In phylogenetic trees, Bayesian posterior probabilities (BPP) from BI analyses/ultra-fast bootstrap supports (UFB) from ML analyses were noted beside nodes. The scale bar represents 0.02 nucleotide substitutions per site; **C.** ND2-based mitochondrial haplotype network.

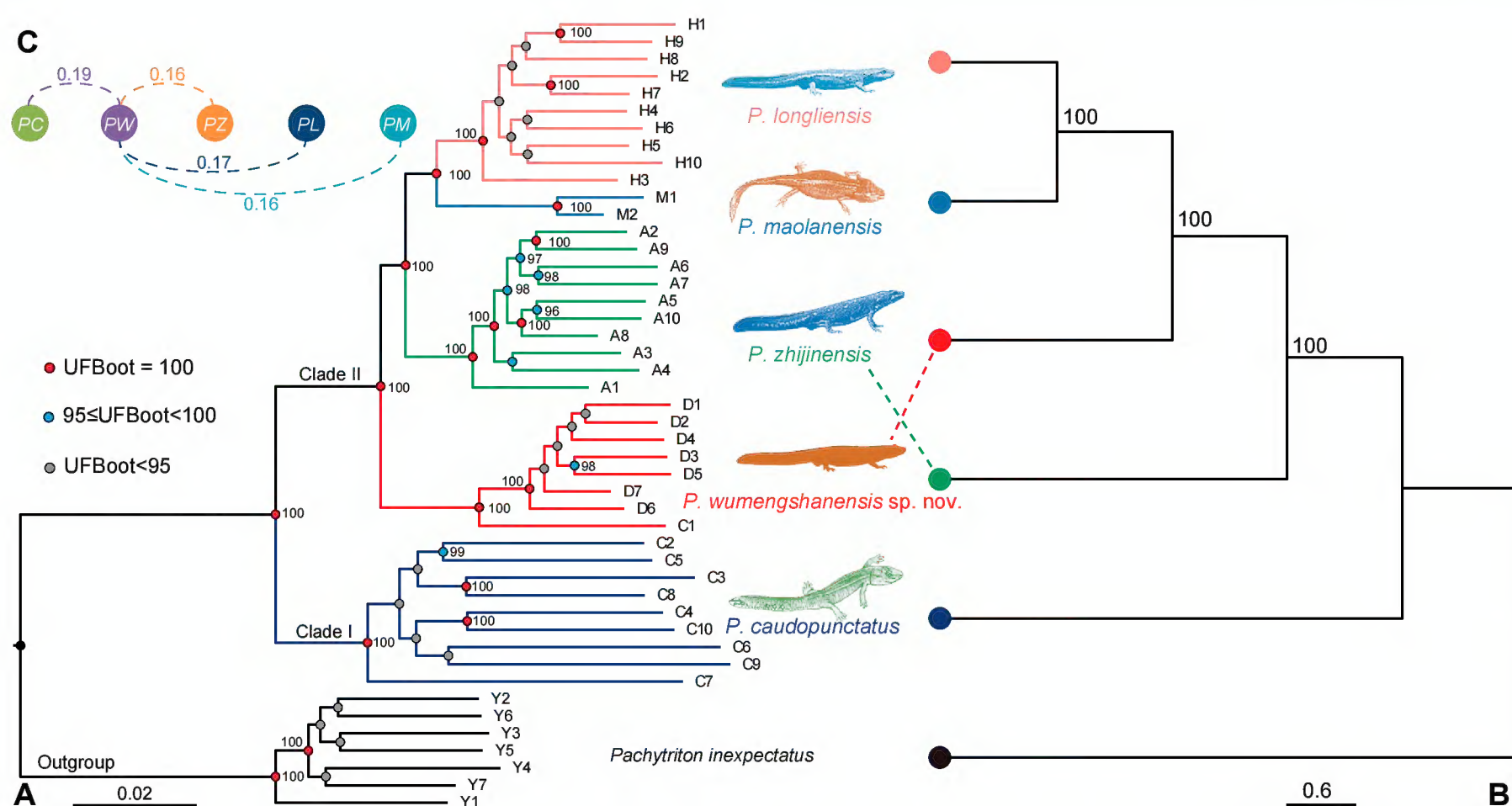


Figure 4. Phylogeny and interspecific differentiation. Maximum likelihood tree reconstructed (A) and species tree generated from SVDquartets (B) analysis based on 1,475,246 SNPs. Support values are displayed on nodes, and dashed lines indicate topology-conflicting species. Tip labels: branch tip numbers are sample numbers for the corresponding species. Note: Sample C1 represents the new species but retains its original label due to initial misidentification and for consistency with NCBI records. (C) Species differentiation between new species and other species. The numbers above and below the dashed line represent *Fst* values. Abbreviation: PC = *P. caudopunctatus*, photo modified from Fei et al. (2006); PW = *P. wumengshanensis* sp. nov.; PZ = *P. zhijinensis*; PL = *P. longliensis*; PM = *P. maolanensis*, photo modified from Gu et al. (2012).

from its closely related congeners, with *Fst* values ranging from 0.16 to 0.19 (Fig. 4C), suggesting partial but significant species divergence.

Based on morphological, mitochondrial, and nuclear genetic differences, there is clear support that the *Paramesotriton* geographic population from Dafang, Guizhou, China, should be treated as a new species.

Taxonomic account

Paramesotriton wumengshanensis Luo & Zhou, sp. nov.

<https://zoobank.org/72A9ACFC-B93C-4727-BA3F-ACA804B7B041>

Figs 5, 6, Suppl. material 1

Chresonymy. *Paramesotriton* sp2 (DF): Luo et al. 2021 (Dafang, Guizhou, China); *Paramesotriton longliensis* (DF): Luo et al. 2022 (Dafang County, Guizhou, China).

Holotype. Adult male, GZNU20180711, collected by Tao Luo on 11 July 2018, in Yuchong Township, Dafang County, Guizhou Province, China, 27.44933332°N, 105.95403671°E, elevation 1137 m a.s.l.

Paratypes. Three adult males: GZNU2018070903, GZNU2018070904, and GZNU2018070905. Three adult females: GZNU2018070901, GZNU2018070902, and GZNU20180709011. The locality and date of all paratypes are same as holotype.

Etymology. The specific epithet *wumengshanensis* refers to the type locality, located within the Wumeng Mountains, Guizhou, China. The suggested English name is the Wumeng Mountains Warty Newt, and the Chinese name is Wū Mēng Shān Luǒ Yuán (乌蒙山瘰螈).

Diagnosis. (1) large body size, TOL 95.5–123.6 mm in adult males, TOL 150.1–160.0 mm in adult females; (2) absence of distinct colored stripes on dorsolateral ridges, consistent with body coloration; (3) absence of vestigial gills and gill filaments; (4) external eyes normal, eye diameter 14–20% of head length; (5) absence of colored spots on the tails of males, a single red-orange stripe from the posterior margin of the cloaca to the 4/5 parts of tail on ventral side; (6) rough skin, body warts rough and large, and longitudinal grooves distinct on the body flanks; (7) yellow spot below the posterior margin of the eye absent; (8) vomerine teeth distinct, middle part depressed outward, having a slightly wider and shallow groove at middle base of each choana, and a distinctly prominent bony plate is present posterior to the choana; (9) anterior end of the epibranchial bone of the hyoid apparatus is near T-shaped.

Description of the holotype. Adult male, TOL 123.6 mm, SVL 72.6 mm. Measurements are presented in Suppl. material 1. Body relatively slender. Head flat, distinctly longer than wide (length/width = 1.4), distinct scent glands on side of head; knob-like (Fig. 6D), with a fleshy protuberance present in the branchial region. Snout short; snout aris evident, truncated, extending beyond lower lip; snout length distinctly longer than eye diameter; nostrils short, close to snout tip; eyes large; labial fold developed, extending from lower eye to tip of snout; gular fold developed; glandular ridge on each side of the head, slightly developed; tongue short, elliptical, both lateral sides dissociated; one premaxilla with tiny teeth on the upper and lower jaws. Vomerine teeth distinct, A-shaped, middle part depressed outward; dentition slowly compressed from posterior to anterior-to-anterior margin



Figure 5. Holotype GZNU20180711 (A–C) and female paratype GZNU20180709011 (D–F) of *Paramesotriton wumengshanensis* sp. nov. in preservative. (A, D) Lateral view. (B, E) Dorsolateral view. (C, F) Ventral view.

meeting between two nares; having a slightly wider and shallow groove at middle base of each choana; a distinctly prominent bony plate is present posterior to the choana (Fig. 7A). Anterior end of the epibranchial bone of the hyoid apparatus is near T-shaped, connected with the first and second ceratobranchials of the bone; ceratohyal knife-like, with the anterior two-thirds in cartilage, left and right unconnected (Fig. 7D). Vertebral ridge developed, light tan, conspicuous, unintermittent, from occiput to tail. Forelimbs short, reaching to tip of snout when adpressed forward; hind limbs longer and sturdier than forelimbs; palm and sole overlap when adpressed forelimb backward and hind limb forward along body flank; without metacarpal or metatarsal tubercles; four fingers and five toes without webbing or fringe; relative finger length $1 < 4 < 2 < 3$; relative toe length $1 < 5 < 2 < 4 < 3$. Tail relatively long; dorsal and ventral caudal fins evident, expanding on posterior half of tail; tail-tip rounded. Cloaca a large slit, swollen into a small mound, villous mastoid around cloaca, many pubes on gaps of cloaca. Skin rough, especially on the back of the body; many warts and granular glands on dorsal skin, dorsal surface of the limbs, and lateral sides of tail. Longitudinal flank grooves distinct on the body flanks, approximately 17 (Fig. 6E). Ventral skin wrinkled, densely covered with small warts (Fig. 6C, H).

Sexual dimorphism. Apophysis of cloaca of the male large and low, and the counterpart of the female small and high (Fig. 5C, F). Female cloaca small and oval without gland in inside and lack finger-like papillae on the cloacal wall.

Coloration. In life, dorsal surface of body black-brown and light olive color. Head, body, and ventral surfaces of limbs dark brown overall with irregular reddish-orange markings. Absence of distinct colored stripes on dorsolateral ridges, consistent with body coloration.

A single red-orange or tangerine yellow stripe extends from the posterior margin of cloaca to the 4/5 parts of the tail on the ventral side (Fig. 6H). Finger and toe tips grayish brown. Color lightens in 75% ethanol preservation.

Comparison. The new species can be assigned to the *P. caudopunctatus* group on the basis of phylogeny and can be distinguished from other species within that group by the following morphological characteristics (Table 4).

Paramesotriton wumengshanensis sp. nov. differs from *P. caudopunctatus* and *P. wulingensis* by absence of colored spots on the tails of males and colored stripes on dorsolateral ridges (vs. presence of purplish red spots on the tails of males and three yellowish brown longitudinal stripes very distinct on dorsolateral ridges).

Paramesotriton wumengshanensis sp. nov. differs from *P. malipoensis* by absence of vestigial gills and gill filaments in adults (vs. presence), snout-vent length greater than tail length (vs. almost equal), and absence of distinct colored stripes on dorsolateral ridges, consistent with body coloration (vs. dorsolateral ridges scattered with distinctive orange-red or brownish-yellow spots).

Paramesotriton wumengshanensis sp. nov. differs from *P. zhijinensis* by absence of distinct colored stripes on dorsolateral ridges, consistent with body coloration (vs. two or three near-continuous yellow stripes on the dorsolateral ridge), yellow spot below the posterior margin of the eye absent (vs. present), absence of vestigial gills and gill filaments in adults (vs. neoteny is common with most adult specimens having vestigial gills and gill filaments), vomerine teeth distinct, middle protruding outward, dentition slowly compressed from posterior to anterior to anterior margin meeting between two nares, having a slightly wider and shallow groove at middle base



Figure 6. Holotype GZNU20180711 (A–E) and female paratype GZNU2018070901 (F–H) of *Paramesotriton wumengshanensis* sp. nov. in life. (A, F) Dorsolateral view; (B, G) Lateral view; (C, H) Ventral view. (D) Lateral view of the head of GZNU20180711 in life; the white arrow indicates the scent gland. (E) Dorsal view of the middle of the body (GZNU20180711), numbers 1–17 denote longitudinal grooves. (I) An individual with a lack of left hind limbs. (J) Habitat.

of each choana, a distinctly prominent bony plate is present posterior to the choana (vs. vomerine teeth indistinct, middle compressed inward, dentition rapidly compressed from posterior to anterior to anterior margin no meeting between two nares, having a small, narrow, deep groove

at middle base of each choana, and bony plate posterior to the choana is highly reduced and indistinct), and ceratohyal knife-like (vs. boat-shaped) (Fig. 7A, B).
Paramesotriton wumengshanensis sp. nov. differs from *P. longliensis* by absence of distinct colored stripes

Table 4. The diagnostic characters selected for the newly described species in this study and the six species of the *P. caudopunctatus* group follow Fei and Ye (2016). Grey shading indicates a clear difference in characteristics compared to that of *P. wumengshanensis* sp. nov.

Species	<i>P. wumengshanensis</i> sp. nov.	<i>P. caudopunctatus</i>	<i>P. wulingensis</i>	<i>P. malipoensis</i>	<i>P. zhijinensis</i>	<i>P. longliensis</i>	<i>P. maolanensis</i>
Eyes	Normal	Normal	Normal	Normal	Normal	Normal	Reduced
Skin	Rough	Rough	Rough	Rough	Rough	Rough	Smooth
Purplish red spots on the tails of males	Absent	Present	Present	Absent	Absent	Absent	Absent
Lateral fringes	Absent	Wide	Wide	Absent	Absent	Absent	Absent
Colored stripes on dorsolateral ridges	Absent	Very distinct	Indistinct or distinct	Absent	Absent	Absent	Absent
Vestigial gills and gill filaments	Absent	Absent	Absent	Present	Present or Absent	Present or Absent	Absent
Anterior end of the epibranchial bone	T-shaped	T-shaped	T-shaped	T-shaped	T-shaped	T-shaped	Y-shaped
Bony plate posterior to the choana	Distinctly prominent and development	Highly reduced and indistinct	Highly reduced and indistinct	NA	Highly reduced and indistinct	Highly reduced and indistinct	Highly reduced and indistinct
Vomerine teeth	Distinct, middle protruding outward; dentition slowly compressed from posterior to anterior margin	Distinct, anterior-middle portion of the dentition is nearly parallel	Distinct, middle compressed inward; dentition rapidly compressed from posterior to anterior margin	NA	Distinct, indistinct, middle compressed inward, dentition rapidly compressed from posterior to anterior margin	Indistinct, middle compressed inward, dentition rapidly compressed from posterior to anterior margin	Distinct, middle compressed inward, dentition rapidly compressed from posterior to anterior margin

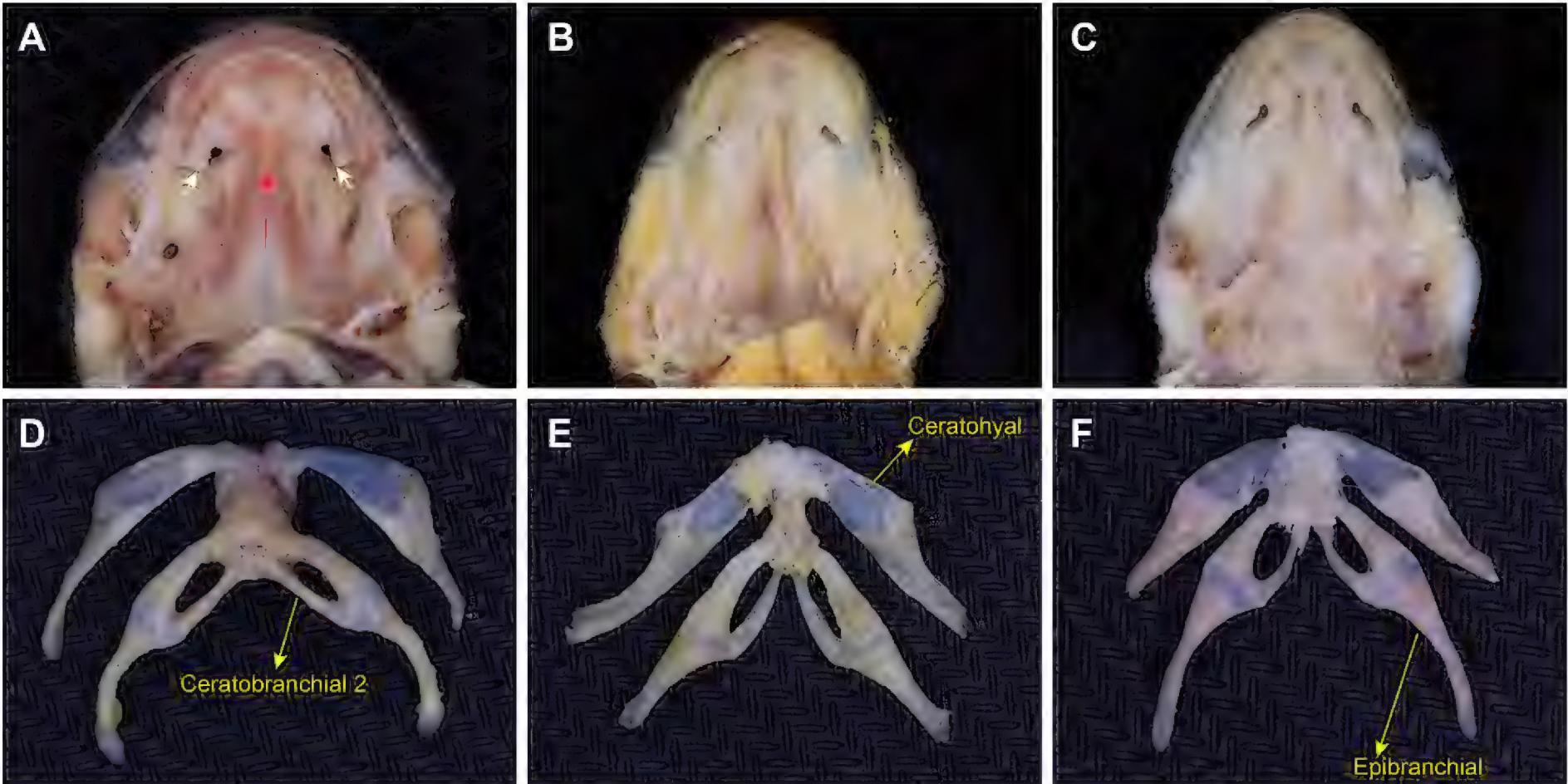


Figure 7. Vomerine tooth morphology (A–C) and structure of hyoid apparatus (D–F) of *P. wumengshanensis* sp. nov. and the other two species in the *P. caudopunctatus* group. (A, D) *P. wumengshanensis* sp. nov. (B, E) *P. zhijinensis*. (C, F) *P. longliensis*. The white arrow in Figure A indicates the bony plate posterior to the choana. The red arrow points to the groove anterior to the vomerine tooth.

on dorsolateral ridges, consistent with body coloration (vs. dorsolateral ridges scattered with distinctive yellow granules or two or three near-continuous yellow stripes on the dorsolateral ridge), yellow spot below the posterior margin of the eye absent (vs. present); vomerine teeth distinct, middle protruding outward, dentition slowly compressed from posterior to anterior to anterior margin meeting between two nares, having a slightly wider and shallow groove at middle base of each choana, a distinctly prominent bony plate is present posterior to the choana (vs. vomerine teeth indistinct, middle compressed inward, dentition rapidly compressed from posterior to anterior to anterior margin no meeting between two

nares, having a small and narrow groove at middle base of each choana, and bony plate posterior to the choana is highly reduced and indistinct), and ceratohyal knife-like (vs. boat-shaped) (Fig. 7A, C).
Paramesotriton wumengshanensis sp. nov. differs from *P. maolanensis* by rough skin and external eyes normal, eye diameter 14–20% of head length (vs. smooth skin and external eyes largely reduced), distinct longitudinal flank grooves (vs. indistinct or absent), and vertebral ridge well-developed and consistent with body coloration (vs. vertebral ridge slightly bulged, yellow) (Fig. 8), and anterior end of the epibranchial bone of the hyoid apparatus is near T-shaped (vs. Y-shaped).



Figure 8. Dorsal and ventral views of five species of the genus *Paramesotriton* distributed in Guizhou. **A.** *P. caudopunctatus*; **B.** *P. wulingensis*; **C.** *P. zhijinensis*; **D.** *P. longlisis*; **E.** *P. wumengshanensis* sp. nov.; **F.** *P. maolanensis* (modified from Gu et al. 2012).

Distribution and habitat ecology. Currently, *Paramesotriton wumengshanensis* sp. nov. is found only in three deep-water pools in the type locality. This area has a mean annual temperature of approximately 20 °C and receives about 1,800 mm of annual precipitation, with a humid subtropical monsoon climate. Surrounding the waterholes are bamboo forests and local crops, including rice, corn, and chili peppers. During a survey in July 2018, several other species were also recorded at the type

locality, including *Triplophysa wulongensis* Chen, Sher-aliev, Shu & Peng, 2021; *Rhinogobius giurinus* Rutter, 1897; *Tylototriton kweichowensis* Fang & Chang, 1932; *Boulengerophrys qianbeiensis* (Su, Shi, Wu, Li, Yao, Wang & Li, 2020); *Oreolalax rhodostigmatus* Hu & Fei, 1979 (tadpoles); *Hyla annectans* (Jerdon, 1870); and *Odorrana grahami* (Boulenger, 1917). The primary threat to the species comes from ongoing mineral development in the upper reaches of the rivers within its range, which has

caused the water in the mainstem rivers to become discolored (blackened), thus restricting the species to deep pools on the riverbanks. Currently, there have been no reports of illegal human capture. However, more ecological and reproductive information on this species is still lacking.

Discussion

In an era of accelerated species extinction (Ceballos et al. 2020), the identification and description of unrecognized species have become an urgent priority for biodiversity conservation. Advances in DNA barcoding (Hebert et al. 2003) have revolutionized taxonomy, enabling the discovery of numerous cryptic species (Xu and Che 2019; Womack et al. 2022) and significantly expanding our understanding of global diversity. In this study, we describe a new species from Dafang, Guizhou, supported by multiple lines of evidence. Morphologically, the species exhibits diagnostic characters distinguishing it from congeners (see Comparison). Genetically, both mitochondrial and nuclear data consistently resolve it as a distinct lineage (Figs 3, 4), with unique haplotypes (Fig. 3C) confirming its evolutionary independence. Geographically, the new species and its closest relatives occupy disjunct ranges separated by major river systems (e.g., the Wujiang and Zhangjiang rivers; Fig. 1). These rivers likely function as biogeographic barriers driving allopatric speciation, with no evidence of current or future gene flow. Collectively, these data unequivocally support its recognition as a new phylogenetic species.

From a conservation biology perspective, clarifying the taxonomic status of *P. wumengshanensis* sp. nov., *P. maolanensis*, *P. longliensis*, and *P. zhijinensis* holds significant practical value. Based on their natural distributions, only the ranges of the first two species are currently covered by protected areas (Gu et al. 2012; this study), while *P. longliensis* and *P. zhijinensis*, which remain in conservation gaps, are listed as Vulnerable and Endangered, respectively, in the IUCN Red List (IUCN 2025). The confirmation of their species status may directly promote differentiated conservation policies for these taxa. First, habitat protection plans can be formulated based on their unique ecological requirements, such as the obligate dependence of *P. maolanensis* on karst cave aquatic systems and the restriction of *P. zhijinensis* to its type locality (Li et al. 2008; Gu et al. 2012). Second, corresponding endangered status assessments should be conducted based on population size, such as the restricted distributions and small populations of *P. wumengshanensis* sp. nov., *P. maolanensis*, and *P. zhijinensis* (Li et al. 2008; Gu et al. 2012; Zhao et al. 2012). Finally, targeted mitigation measures can be implemented against species-specific threats, such as agricultural runoff and domestic water pollution in the distribution areas of *P. longliensis* and *P. zhijinensis* (Zhao et al. 2012; this study). Such a species-based conservation framework not only enhances biodiversity inventories but also provides a scientific foundation for implementing species-specific conservation planning.

Historical gene flow events may have obscured species boundaries. Mitochondrial DNA (mtDNA), which is maternally inherited, can be dominated by the mtDNA of one lineage during gene flow events, leading to minimal mitochondrial divergence and cytonuclear discordance. For example, Yuan et al. (2022) revealed that frequent historical mitochondrial introgression resulted in small genetic distances (ranging from 0.3% to 0.8%) among the three species of the *O. grahami* complex (*O. grahami*, *O. junlianensis*, and an undefined species). Similar phenomena have been observed in Asian black bears (Zou et al. 2022), primates (Rivas-González et al. 2023), bats (Liu et al. 2024), and carnivores (Santos et al. 2025). Therefore, the cytonuclear discordance observed within the *P. caudopunctatus* group may stem from historical gene flow and/or incomplete lineage sorting (Sloan et al. 2017; Luo 2024). Future comprehensive analyses based on genomic evidence will be needed to investigate their respective contributions.

Acknowledgments

This research was supported by the following programs: the 2024 National Key Protected Wildlife Resources Survey in Xishui County, Guizhou Province, China (11904/0624063), the 2024 National Key Protected Wildlife Resources Survey in the Chishui River Basin, Guizhou (P52000020240003VQ), the Endangerment Processes and Mechanisms of Endangered Flagship Animals (2022YFF1301502), the Guizhou Normal University Academic Emerging Talent Fund Project (Qianshi Xin Miao [2021] 20), and the Application of Amphibian Natural Antioxidant Peptides as Cosmetic Raw Material Antioxidants (QKZYD [2020]4002). We extend our sincere gratitude to Ms. Huan He for her invaluable assistance in sample collection. She accompanied the first author to Huishui County, Guizhou Province in June 2018 to collect samples of the genus *Paramesotriton*. We thank Chang-Ting Lan and Ming-Le Mao for their help and guidance during the photography process. Finally, we acknowledge LetPub (www.letpub.com) for their linguistic assistance during manuscript preparation.

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Supplementary material 1

Morphological measurement data of the new species *P. wumengshanensis* sp. nov., *P. longliensis*, and *P. zhijinensis*

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Explanation note: Text.

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Supplementary material 2

Results and percentage of variance explained by principal component and discriminant function analyses

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